volved in the upregulation of MMPs and angiogenic factors, and in some but not all fibroblast cell lines, H-ras transfection resulted in high heparanase activities, which correlated with metastatic potential[11]. Moreover, as heparanase and MMP-9 co-localize in neutrophil tertiary granules[12], it will be interesting to determine whether these molecules are coordinately regulated by upstream signaling pathways, or whether they provide alternative or complementary functions in different cellular contexts.

In addition to the correlative studies reported by both groups here[1,2], Vlodavsky *et al.*[1] show that transfection of the heparanase gene enhanced the metastatic potential of rodent tumor cells, providing direct evidence for a role in invasion. The finding of heparanase activity in the urine of some cancer patients is also important, as it may allow assessment of disease progression or response to therapy. Heparanase inhibitors (mainly based on heparin and similar polysaccharides) have been shown to inhibit tumor growth and/or metastasis, angiogenesis and vascular damage in some cases in experimental models. The availability of large quantities of recombinant enzyme and sensitive functional assays will facilitate the design and testing of better and more selective inhibitors. However, as with many enzyme systems, balance is essential, and the finding that bFGF signaling is facilitated when bound to cell surface HS cautions that use of heparanase inhibitors may shift the balance from free bFGF to HS-bFGF, alter recycling and degradation pathways and enhance rather than inhibit cellular activation. Careful evaluation of possible adverse effects on normal physiological functions will also be imperative.

In summary, the elucidation of the nucleotide and amino acid sequence of the first mammalian heparanase represents an important achievement that removes barriers to the better understanding of its role not only in metastasis, but also in inflammatory and autoimmune conditions.

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# MEK Wars, a new front in the battle against cancer

**A specific inhibitor of the mitogen-activated protein kinase (MAPK or ERK) pathway is introduced as a new member in the growing search for cytostatic drugs that block tumor growth (pages 810–816).**

NICHOLAS S. DUESBERY[1], CRAIG P. WEBB[2] & GEORGE F. VANDE WOUDE[3]

TUMOR CELLS PROLIFERATE and spread throughout the body in apparent disregard of normal environmental cues. Many of the genes that allow tumor cells to bypass this regulation have been discovered; these represent targets for drug therapy. The new challenge is to treat tumor cells so that they once again respond normally to environmental cues. On page 810 of this issue, Sebolt-Leopold *et al.* demonstrate that by inhibiting the mitogen-activated protein kinase (MAPK or ERK) signal transduction pathway, tumor cells revert to a nontransformed phenotype *in vitro*, and tumor growth *in vivo* is arrested[1].

External stimuli acting at the cell surface trigger a cascade of intracellular signals that can lead to proliferation or differentiation. Elucidating the multitude of biochemical pathways that transduce extracellular signals from the cell surface to precipitate rapid and profound cellular responses has been a research area of extraordinary discovery in the last decade. MAPK is essential in one such signaling pathway (Fig. 1). Extracellular growth factors bind to specific cell surface receptors, causing changes in their conformation and, in some cases, such as with the tyrosine kinase receptors, inducing intrinsic enzymatic activity. This activity leads to the recruitment and activation of proteins like Ras and Raf at the inner surface of the cell membrane, resulting in a series of phosphorylation events and sequential activation of MAPK kinase 1 (MAPKK1 or MEK1) and MAPK (ERK1/2). MAPK phosphorylates a multitude of downstream substrates involved in a multitude of cellular responses from cytoskeletal changes to gene transcription (reviewed in ref. 2). How is this signaling pathway involved in the etiology of human tumors? Conditional MAPK activation is important in gene regulation, promoting G1 cell cycle progression before DNA replication, as well as in spindle assembly during both meiotic and mitotic cell division (Fig. 1). However, many oncogenes constitutively activate the MAPK pathway, and it is this inappropriate activation that mediates the transformed phenotype (reviewed in ref. 2). Thus, MEK1, which has been engineered to be constitutively activated, can 'transform' mammalian cells to a cancerous phenotype[3–5], whereas inhibition of MAPK activity can inhibit the growth of Ras-transformed cells *in vitro*[6–9]. In addition, activated MAPK or increased levels of MAPK expression have been detected in a variety of human tumors including breast carcinoma and glioblastoma, as well as primary tumors derived from kidney, colon, and lung tissues[10–13]. Thus, inappropriate activation of the MAPK pathway is an essential feature common to many types of

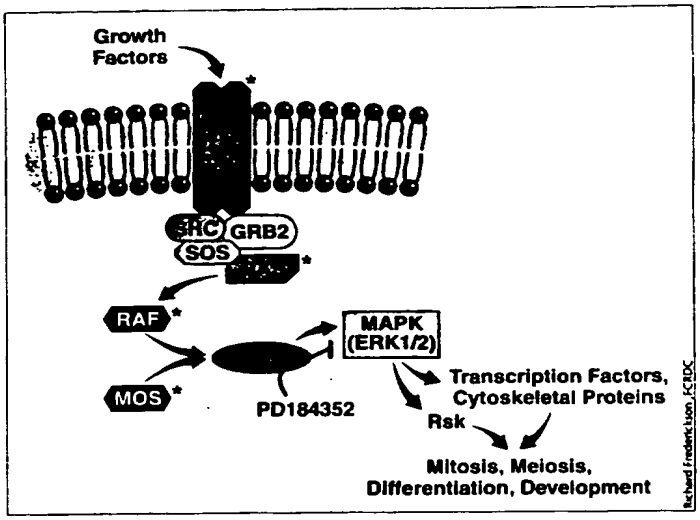


**Fig. 1** Intracellular signaling pathways that mediate MAPK (ERK1/2) activation after growth factor stimulation. Growth factors such as epidermal growth factor, hepatocyte growth factor/scatter factor and platelet-derived growth factor bind to their specific cell surface receptor with intrinsic tyrosine kinase activity. After ligand binding, adjacent receptors are activated through dimerization, resulting in transphosphorylation on tyrosine residues. This promotes SH2-type (phosphorylation-dependent) interactions with a number of downstream effectors, including the adapter proteins Grb2 and SHC. Grb2 and SHC mediate the activation of the Ras GTP-binding protein, through association with the Son-of-Sevenless GTP-GDP exchange factor (SOS). GTP-bound Ras recruits the Raf-1 Ser/Thr kinase to the inner surface of the plasma membrane, where it is able to phosphorylate, and thus activate, MEK1/2. Similarly, the Mos kinase is able to phosphorylate and activate MEK directly. MEK1/2 is a dual-specificity kinase that phosphorylates MAPK (ERK1/2) on both Ser/Thr and Tyr amino acids, resulting in MAPK activation. After being activated, MAPK interacts with many substrates, mediating their phosphorylation and in many cases, altering their function. For example, the ribosomal S-6 kinase (Rsk) is phosphorylated by MAPK, as are many cytoskeletal proteins, including essential components of microtubules and the actin cytoskeleton. In addition, phosphorylated MAPK translocates to the cell nucleus, where it regulates the activity of a number of transcription factors such as Elk-1, thereby modulating gene transcription and expression. The combined effects of these events are manifested by alterations in cell behavior, typified by long-term effects on cell proliferation and/or differentiation, as well as more transient cellular effects. Asterisks identify members of this pathway that can be activated as oncogenes.

tumors; and molecules, such as MEK, participating in this signaling pathway are potential targets for cancer therapy.

In this issue, Sebolt-Leopold *et al.*[1] characterize the properties of a new inhibitor of MEK1, PD 184352. Unlike previously characterized MEK inhibitors[9-14], PD 184352 specifically inhibits MEK1 and, at relatively low concentrations, prevents MAPK activation in a variety of colon, cervical, pancreatic and breast carcinoma cells. Consistent with the known properties of the MAPK pathway, PD 184352 inhibited the growth of some tumor-derived cell lines *in vitro*. This seemed to be partly a cell cycle effect, as treated cells accumulated at the G1 phase of the cell cycle, before DNA replication. Moreover, treatment of transformed cells with PD 184352 caused reversion of the transformed cell phenotype: the cells assumed a flattened morphology and lost their ability to grow in the absence of a substrate. Although this in itself is of great interest, the *in vivo* data are the most remarkable.

To assess the effect of this drug on MAPK activity *in vivo*, PD 184352 was administered orally or injected intraperitoneally into mice with implanted human ovarian and colon xenograft tumors. When these tumors were subsequently excised and assayed, they showed a profound decrease in activated MAPK. The authors also show that PD 184352 considerably inhibited the growth of implanted tumors, both when administered on the day after the tumor cell inoculation and when applied after tumor development. In addition to demonstrating the role of MAPK in cell proliferation, Sebolt-Leopold *et al.*[1] show that, *in vitro*, PD 184352 inhibits hepatocyte growth factor/scatter factor-mediated tumor cell invasiveness. Thus, in addition to the antiproliferative effects of PD 184352, its anti-invasive properties may also contribute to a reduction in tumor progression. These results clearly demonstrate that inhibition of the MAPK pathway can impede the growth of human tumor xenografts *in vivo*, and show the importance of this pathway as a target in the war against cancer. The MAPK pathway has also been shown to be important in an experimental metastasis model system[4]. This drug will allow direct testing of whether inhibiting the MAPK pathway alone will stem invasion and metastasis. PD 184352 now joins a growing list of agents that are cytostatic for tumor growth, such as farnesyl transferase inhibitors and inhibitors of angiogenesis, but is directed against a new target. However, given the ubiquitous role of the MAPK pathway in normal cell signaling, from immune responses and neuronal function to oocyte maturation[2], it will be important to assess potential undesirable effects of MEK inhibitors on normal physiological processes.


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